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Application Note 01117

Fast and Sensitive EtG Analysis in Urine With Pursuit™ C18 HPLC Column and the 320-MS LC/MS/MS

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Introduction

β-D-ethyl glucuronide (EtG) is a minor metabolite of ethanol (ethyl alcohol). It is formed *in vivo* as a consequence of alcohol consumption. A small fraction (0.02%) of a dose of alcohol is conjugated in the liver with glucuronic acid to form ethyl glucuronide. This compound is excreted in the urine.

EtG can be detected in the blood for up to 36 hours and in the urine for up to 5 days after heavy alcohol use. A positive finding of EtG in urine of an individual provides strong evidence that the person recently consumed alcohol, even if the alcohol itself is no longer detectable. Therefore, this compound is regarded as a biomarker of alcohol consumption and, potentially, relapses of alcohol use by an individual who is under restriction. EtG analysis is rapidly being accepted by state probation departments, criminal courts, and schools.

Varian Pursuit C18 HPLC columns can provide good and fast separation of EtG from urine samples. By means of 320-MS LC/MS/MS system, the method demonstrated good linearity, high sensitivity, and stable ion ratios for identification. Detection limits of 10 ppb can be easily reached while the reporting limit is 52 ppb (LOD) and 152 ppb (LOQ) (1).

Instrumentation

- Varian 320-MS LC/MS/MS
- Varian 212-LC Binary Gradient LC/MS Pumps
- Varian Combi PAL AutoSampler

Materials and Reagents

Samples: EtG and D5-EtG were purchased from Lipomed.

Standard stock solutions of EtG were prepared in CH_3OH/H_2O (5%/95%) at 1.0 mg/mL concentration. Internal standard solution was prepared in CH_3OH/H_2O (5%/95%) at 0.1 mg/mL concentration.

A serial dilution of standard solution was performed to get 100 ppm, 10 ppm, 1 ppm, 100 ppb, 50 ppb and 10 ppb spiked with internal standard. Matrix test was performed with EtG spiked in urine, which was then diluted to different concentrations with urine.

HPLC Conditions

Column: Pursuit™ C18 5 µm, 50 x 4.6 mm ID (Varian Part No. A3000050X046)

Guard Column: Polaris™ C18-A 5 µm, 10 x 4.6 mm ID (Varian Part No. A2000010D046)

Mobile Phase: A: H_2O + 0.1% HCOOH
B: CH_3CN
A:B - 95:5 (isocratic)

Flow Rate: 0.7 mL/min

Temperature: Ambient

Results & Discussion

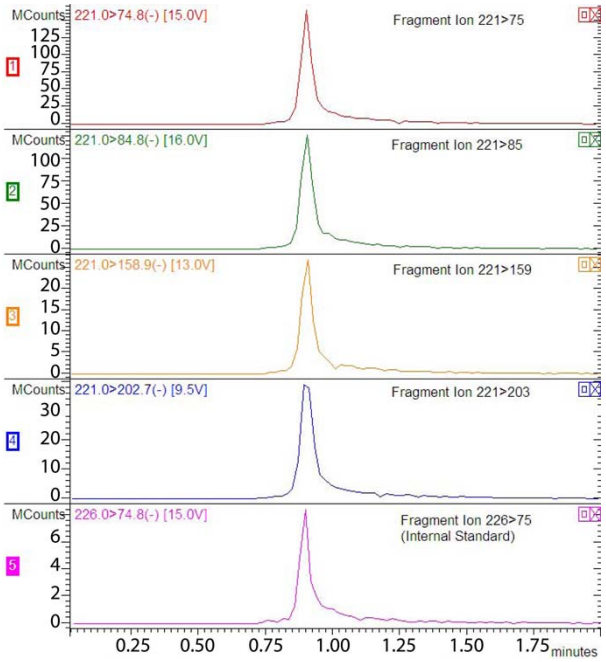

Figure 1 Fragment ions of EtG that could be used for quantitaion. From top to bottom: 221 → 75, 221 → 85, 221 → 159, 221 → 203, 226 → 75 (D5-EtG, IS).

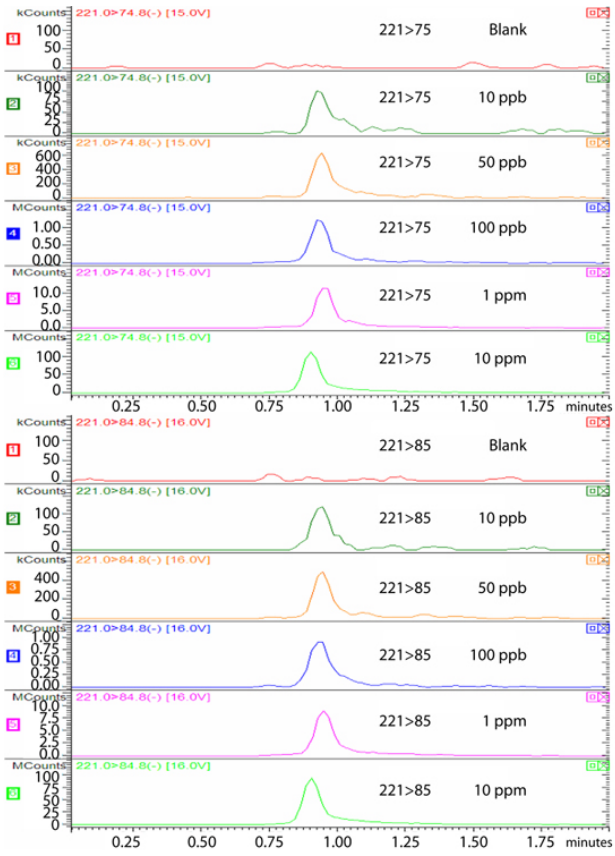

Figure 2 Quantitation of spiked EtG in urine from 10 ppb to 10 ppm for ion ratios, 221 → 75 (top panel), and 221 → 85 (bottom panel).

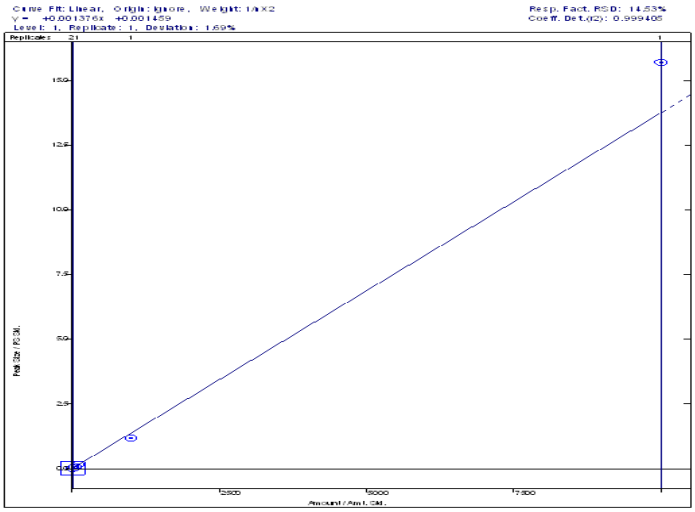

Figure 3 Calibration curve for EtG (221→ 85) from 10 ppb to 10 ppm.

Discussion

This analysis was a mimic of the "direct injection" procedure typically adopted by forensic labs for EtG analysis in urine samples. The "direct injection" involves spiking the internal standard in urine, and diluting 10-fold without any extraction.

To confirm and quantitate EtG, a couple of different ratios of fragment ions could be used, such as 221 → 75, 221 → 85, 221 → 159, 221 → 203, depending on the QC/QA requirements. The sensitivity of the fragment ions was different (Figure 1). Figure 2 shows an overlay of quantitation of spiked EtG in urine from 10 ppb to 10 ppm levels for two ion ratios. The separation is achieved within 2 min. Pursuit™ C18 column yields fast separations as a result of the reduced hydrophobicity characteristic of the Pursuit family, created by combining advanced bonding technologies on an ultra high purity silica. In this matrix, all other compounds were separated by the column and diverted to waste. The interferences of matrix were minimized. Figure 3 displays a 5-point calibration curve showing good linearity from 10 ppb to 10 ppm, with r^2 greater than 0.999.

Conclusion

A fast, efficient, cost-effective, and sensitive method for EtG analysis in urine has been developed using Pursuit C18 column and 320-MS LC/MS/MS detector, which offer speed and sensitivity respectively.

References

1. Wurst, F. M.; Skipper, G. E.; & Weinmann, W. "Confirmatory Analysis of Ethylglucuronide in Urine by Liquid-Chromatography/Electrospray Ionization/Tandem Mass Spectrometry According to Forensic Guidelines", *Journal of The American Society for Mass Spectrometry*, **15,** 188-193, (2004).

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